


Epidemiological Aspect of Bacteremia Cases in Hyperthermic Patients Hospitalized at the Niamey General Reference Hospital, Niger

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Abstract

Introduction: Bacteraemia is a serious infection that is responsible for high morbidity and mortality worldwide through the use of central or peripheral venous catheters. **Material and Method:** This was a prospective descriptive cross-sectional study of patients hospitalized at the Niamey General Reference Hospital. Patient blood was collected during a thermal peak and inoculated into FN PLUS, FA PLUS and PF PLUS blood culture bottles according to patient age, and sent to the Microbiology laboratory for analysis. These flasks were introduced into the BactAlerT 3D at 37°C for incubation. Positive flasks were subcultured onto appropriate media for bacterial growth and identification, and tested for antibiotic sensitivity at 0.5 MacFarland by the Kurbi Bauer method. Data were entered into Microsoft Excel and analyzed using SPSS version 16 software. **Result:** A total of 250 patients were included in our study. The mean age of the patients was 45 ± 17 years. The prevalence of bacteremia among patients was 14%. The bacteria encountered were *Staphylococcus aureus* (31.43%), *Escherichia coli* (22.86%), *Klebsiella pneumoniae* (11.43%) and other bacterial species (8.57%). Bacteremia were more common in men, and patients hospitalized in the Neurology, Internal Medicine and Emergency Departments had higher bacteremia levels, at 4.4%, 2.8% and 2.4% respectively.

Staphylococcus aureus strains were sensitive to oxacillin, norfloxacin, levofloxacin (18.18%), erythromycin (72.73%), gentamicin and tetracycline (36, 36%) *Escherichia coli* strains showed sensitivity to amoxicillin (12.50%), amoxicillin/clavulanic acid, piperacillin, ciprofloxacin and amikacin (25%). No *Escherichia coli* strains were sensitive to 3rd-generation cephalosporins, but ertapenem and imipenem were sensitive (100%) to all strains. **Conclusion:** Bacteremia is common, especially in patients with central venous catheters, with a high frequency of *Staphylococcus aureus* and *Escherichia coli*, antibiotic resistance to these isolates is very high in hospitalized patients.

Keywords

Bacteremia, Hyperthermic Patients, Epidemiological Aspect, HGR, Niger

1. Introduction

Bacteremia is a public health problem, especially in patients with central or peripheral venous catheters [1] with a prevalence of 1,200,000 cases each year in Europe [2] and has a high mortality and morbidity rate [2] [3] 30 million deaths worldwide [4]. Data on antibiotic resistance in low-income countries are scarce due to a lack of local or regional epidemiological surveillance [5]. In one study in Niger, bacterial resistance to certain commonly used antibiotics was very high, particularly in cases of community-acquired bacteremia, where resistance was 58% to amoxicillin-clavulanate and 100% to amoxicillin and amoxicillin-clavulanate in nosocomial bacteremia [6]. Mortality linked to antibiotic resistance is estimated at 7.3 deaths per 100,000 bloodstream infections in sub-Saharan Africa [7] with an average economic cost of 18,000 euros per case of bacteremia, depending on the bacterium responsible [8]. This blood-related infection can be considered a healthcare-associated infection. Fecal colonization can serve as a reservoir for transmission and colonization of another person [9]. These micro-organisms enter the bloodstream and cause bacteremia [10]. In low- and middle-income countries, C3Gs, in particular ceftriaxone, are the first-line antibiotic used to treat bacteremia [11]. However, overuse of this antibiotic has led to the emergence of multi-resistant bacteria in the bloodstream [12] and increased treatment failures for blood-related infections [13]. This re-emergence of multi-resistant Enterobacteriaceae remains a major concern, given the upward trend in antibacterial resistance [14]. The prevalence of bacteremia due to multi-resistant enterobacteria has risen from 6.3% to 15.8% in 2022 [15] [16]. However, this type of bacteremia has seen a marked increase in Gram-positive bacteria, particularly *Staphylococcus aureus*, from 1.6% to 3.8% in 2020 [17].

In Africa, and particularly in hospitals in Niger, the overuse of broad-spectrum antibiotics in hyperthermic patients with no signs of bacterial infection is likely to lead to greater antibiotic resistance. To find out, we set out to determine the epidemiological profile of bacteria isolated and their resistance to antibiotics in cases

of bacteremia in hyperthermic patients hospitalized in hospitals.

2. Materials and Methods

2.1. Study Site, Period and Type

The study was carried out in the hospital wards and Microbiology laboratory of the Niamey General Reference Hospital over a 3-year period from 2020 to 2022. This was a prospective, descriptive, cross-sectional study.

2.2. Study Population

Our study population was hyperthermia patients with a temperature greater than or equal to 38°C hospitalized in the wards of the Niamey General Reference Hospital.

2.3. Patient Selection Criteria

During the study period, all patients who presented with a temperature above 37.5°C and who underwent blood sampling by nursing staff at the request of a physician to look for possible bacteremia, were included in the study. Nurses monitored patients' temperatures every 2 hours, using a temperature monitoring form to check for a thermal peak.

2.4. Blood Sampling in Hyperthermic Patients

The patients' blood samples were taken by the nursing staff after lying the patients down in comfortable positions during the thermal peaks, a sampling site was chosen at the elbow folds. After disinfecting the patient's skin at the elbow crease with three 70°C ethanol alcohol swabs, 2 to 3 minutes apart. By venipuncture, using appropriate devices, blood was collected through the opercula, FN PLUS and FA PLUS blood culture bottles for adults and PF PLUS for pediatric patients. After you have finished introducing the patient's blood into the blood culture bottles at the lines indicated on the bottles, the lids of blood culture bottles were disinfected with a 70°C ethanol alcohol buffer.

2.5. Labeling and Transporting Blood Culture Bottles to the Laboratory

Inoculated blood culture bottles were labeled with patients' first and last names, their original origins, date and time of sampling. They were promptly forwarded to the laboratory, accompanied by their correctly completed analysis reports.

2.6. Processing Blood Culture Bottles in the Laboratory

Once the blood culture bottles and test reports had been received in the laboratory, they were numbered, the patient information was recorded in the register, and finally, the blood culture bottles were incubated in the BactAlerT 3D automat at a temperature of 37°C, in accordance with the precautions and handling pro-

cedures of the BactAlerT 3D automat.

2.7. Procedure for Inserting Blood Culture Bottles into the BactAlerT 3D automated System

After pressing the blood culture bottle diagram, which appeared in blue at the bottom of the machine's screen, 5 fields appeared on the automaton to record patient information by filling in the 5 fields below in chronological order: In the first field, the barcode of the blood culture bottles was entered using the barcode reader, in the second field, the blood culture bottle number has been entered, in the third field, the origin of blood culture bottles taken from patients has been entered, in the fourth field, the patient's first name has been entered, and in the fifth field, the patient's last name has been entered. After filling in the 5 required fields, the blood culture bottles were introduced into the BactAlerT 3D automaton, then validated at the end to start incubation and reading of the blood culture bottles (**Figure 1**).



Figure 1. BACT/ALERT 3D (BIOMERIEUX).

This automated method detects the release of CO₂ by reflectometry every 10 minutes for up to 5 days [18] (**Figure 2**).

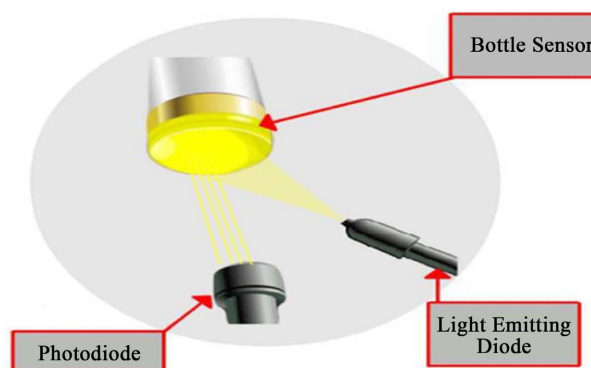


Figure 2. BACT/ALERT 3D light reflection (BIOMERIEUX).

3. Gram Staining Method

First step: After making a smear on a slide with the blood culture broth, cover the smear with crystal violet and allow the dye to stand for one minute, then wash the smear with distilled water and drain the slide. Second step: Cover the smear with lugol solution and allow the dye to stand for one minute, then wash the smear with distilled water and drain the slide. Third step: Decolorize the smear with 96°C alcohol until the waste is colorless, about 15 to 30 seconds depending on the size of the smear, then rinse gently with distilled water and remove excess water from the smear. Fourth step: counterstain the smear with safranin solution and allow the dye to stand for one minute, then wash the smear with distilled water and drain the slide. After drying the slide at room temperature, the smear was observed under the microscope at $\times 100$ objective with immersion oil to read the Gram staining result.

3.1. Isolation of Bacteria on Solid Media

After homogenizing the positive blood culture bottles, the caps of the blood culture bottles were disinfected under a flame with three 70°C alcohol-ethanol swabs before aspirating the broths, blood culture bottle broth samples were taken using needles associated with 1 ml syringes. After performing Gram staining on blood culture broths to differentiate Gram-negative from Gram-positive bacteria. Blood culture broths were inoculated on Columbia gelose culture media, fresh blood gelose, Chocholat, Chapman and Mac Conkey gelose based on Gram stain results. The seeded culture media were incubated at 37°C in an oven for 24 hours.

3.2. Identification of Bacteria Isolated in Positive Blood Cultures

Bacterial identification was based on morphological, cultural and biochemical orientation characteristics such as catalase for gram-positive cocci and oxidase for gram-negative bacilli. For Gram-negative bacteria, the API 10S identification gallery was used to identify enterobacteria and non-fermentative Gram-negative bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

3.3. Oxidase Test

To perform the oxidase test, we placed a blotting paper disc on an object slide, then applied a drop of oxidase reagent to the blotting paper disc. We then used a Pasteur pipette to pick a colony from each bacterial culture (Gram-negative bacilli) and crush it onto the blotting paper disc impregnated with oxidase reagent. A positive reaction is indicated by a blue or purple coloration appearing within 30 seconds. The absence or appearance of a blue coloration after 30 seconds indicates a negative reaction.

3.4. Catalase Test

To perform the catalase test, a drop of hydrogen peroxide reagent was placed on

an object slide, then a bacterial colony was taken from a bacterial culture, introduced and crushed in the hydrogen peroxide reagent. A positive result is indicated by the release of air bubbles in the form of foam.

3.5. Identification of Gram-Negative Bacilli

The API 10S gallery was used to identify Gram-negative bacilli. It is a miniaturized identification gallery that enables 11 biochemical characteristics to be investigated by enzymatic reactions. The API 10S gallery comprises 10 microtubes containing substrates in dehydrated form. After preparing a 0.5 MacFarland bacterial suspension with saline and bacterial colony solution, the microtubes were inoculated with this bacterial suspension and incubated at 37°C in a study for 24 hours. The positive or negative results of the biochemical characters were read as spontaneous color changes or as revealed by the addition of reagents. The sum of the numbers assigned to each character gave codes corresponding to the bacterial species.

3.6. Antibiotic Susceptibility Testing of Bacteria Isolated from Blood Cultures

Antibiotic susceptibility testing was performed using the Muller-Hinton agar disc diffusion method as recommended by CA-SFM/EUCAST 2020 [19]. After swabbing the Muller-Hinton agar plates with a 0.5 MacFarland, prepared in a hemolysis tube containing 3 ml of saline solution to which a bacterial colony has been added. Antibiotic discs were placed on Muller-Hinton agar with forceps, respecting a distance of 30 mm between two antibiotic discs and 15 mm between an antibiotic disc and the plate wall.

3.7. Data Processing and Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 16 software.

3.8. Results

In the present study, 250 blood samples were taken from hospitalized patients during a thermal peak above 37°C. Men accounted for 65.20% versus 34.80% for women, with a sex ratio of 1.87. The average age of patients was 45 ± 17 years.

3.9. Diversity of Enterobacteria Isolated from Blood Samples

The prevalence of bacteremia among patients was 14%. The prevalence of Enterobacteriaceae was 54.28%, followed by Gram-positive cocci and non-fermenting Gram-negative bacilli with 40% and 5.52% respectively. Among bacteria isolated from patients, the leader was *Staphylococcus aureus* with a prevalence of 31.43% (95% CI: 2.22% - 7.74%), followed by *Escherichia coli* with a prevalence of 22.86% (95% CI: 1.39% - 6.21%), *Klebsiella pneumoniae* with a prevalence of 11.43% (95% CI: 0.44% - 4.05%), *Klebsiella oxytoca*, *Enterobacter cloacae*, Salmonella spp, *Aci-*

netobacter baumannii had a prevalence of 5.71% each and finally *Citrobacter freudii*, coagulase-negative Staphylococcus, *Enterococcus faecalis* and *Enterococcus faecium* had a prevalence of 2.86% each (Table 1). Bacteremia was more common in men, with a prevalence of 60% (p-value = 0.11). Patients on the Neurology ward were the most affected by bacteremia, with a prevalence of 4.4%, followed by the Internal Medicine and Emergency departments, with prevalences of 2.8% and 2.4% respectively (Table 2). *Staphylococcus aureus* strains were sensitive to oxacillin, norfloxacin, levofloxacin (18.18%), penicillin (9.09%), ciprofloxacin (27.27%), erythromycin (72.73%), gentamicin and tetracycline (36.36%), and none of these *Staphylococcus aureus* strains were sensitive to tobramycin or vancomycin (Figure 1). *Escherichia coli* strains showed sensitivity to amoxicillin and tobramycin (12.50%), amoxicillin/clavulanic acid, piperacillin, ciprofloxacin and amikacin (25%). Antibiotics such as ticarcillin, ticarcillin/clavulanic acid, 3rd-generation cephalosporins, nalidixic acid and levofloxacin have shown no effective activity against *Escherichia coli* strains (Figure 3). *Klebsiella pneumoniae* strains were sensitive to amoxicillin/clavulanic acid, 3rd-generation cephalosporins, nalidixic acid and levofloxacin (25%). 50% of these strains were sensitive to piperacillin/tazobactam, ciprofloxacin, gentamicin, tobramycin and imipenem. The sensitivity of these *Klebsiella pneumoniae* strains to amikacin was (75%). None of these *Klebsiella pneumoniae* strains were sensitive to ticarcillin, ticarcillin/clavulanic acid, piperacillin or ertapenem (Figure 3, Figure 4).

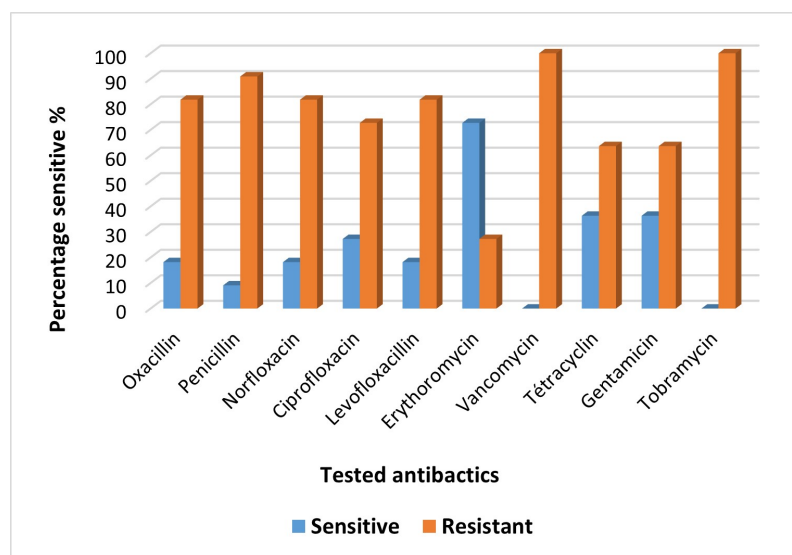


Figure 3. Susceptibility profile of *Staphylococcus aureus* isolates.

4. Discussion

In our study, 250 patients were sampled. Men accounted for 65.20%, compared with 34.80% for women. This was demonstrated in a Tanzanian study on bloodstream infections in 2020 [20]. The average age of patients was 45 ± 17 years. A researcher in Ethiopia has already reported an average age of 47 ± 13.8 in 2021.

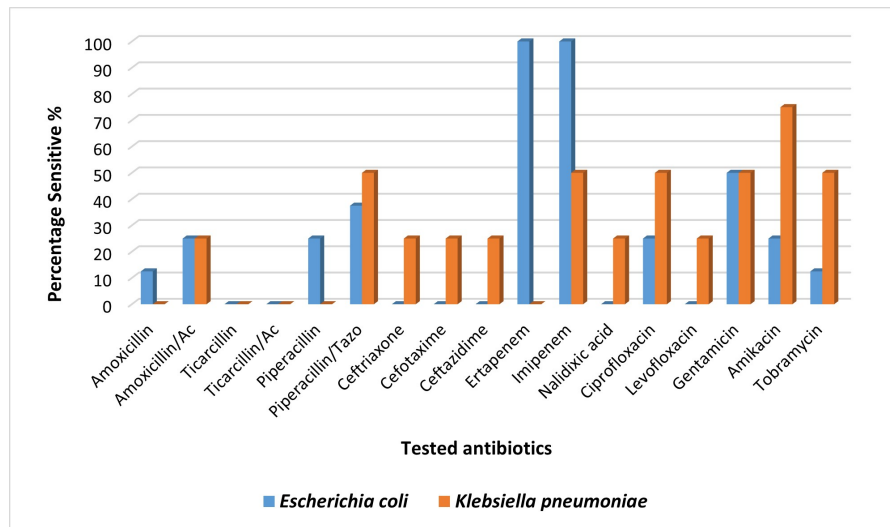


Figure 4. Susceptibility profile of *Escherichia coli* and *Klebsiella pneumoniae* isolates.

Table 1. Distribution of bacteria responsible for bacteremia in patients.

Bacteria identified	Number	Percentage	95% CI
<i>Acinetobacter baumannii</i>	2	5.71	95% CI 0.10% - 2.86%
<i>Citrobacter freundii</i>	1	2.86	95% CI 0.01% - 2.21%
<i>Enterobacter cloacae</i>	2	5.71	95% CI 0.10% - 2.86%
<i>Enterococcus faecalis</i>	1	2.86	95% CI 0.01% - 2.21%
<i>Enterococcus faecium</i>	1	2.86	95% CI 0.01% - 2.21%
<i>Escherichia coli</i>	8	22.86	95% CI 1.39% - 6.21%
<i>Klebsiella oxytoca</i>	2	5.71	95% CI 0.10% - 2.86%
<i>Klebsiella pneumoniae</i>	4	11.43	95% CI 0.44% - 4.05%
<i>Salmonella spp</i>	2	5.71	95% CI 0.10% - 2.86%
<i>Staphylococcus aureus</i>	11	31.43	95% CI 2.22% - 7.74%
<i>Staphylocoque à coagulase négative</i>	1	2.86	95% CI 0.01% - 2.21%

Table 2. Breakdown of bacteremia cases by hospital ward.

Hospitalization department	Positive bacteremia		Negative bacteremia	
	Number	Percentage	Number	Percentage
Cardiology	1	0.4	6	2.4
Visceral Surgery	1	0.4	3	1.2
Endocrinology	3	1.2	12	4.8
Hepato-Gastro-Enterology	1	0.4	2	0.8
Internal Medicine	7	2.8	24	9.6
Nephrology	0	0	12	4.8
Neurosurgery	0	0	9	3.6

Continued

Neurology	11	4.4	33	13.2
VIP Pavilion	1	0.4	0	0
Reanimation	4	1.6	37	14.8
Medical emergencies	6	2.4	77	30.8
Total	35	14	215	86

[21]. The prevalence of bacteremia among patients was 14%. This result was slightly higher than that reported by Habyarimana *et al.* in 2021 at Kigali University Hospital [22]. A meta-analysis study reported a prevalence of 14.6% in Africa [23]. The prevalence of Gram-negative enterobacteria predominated at 54.28%, followed by Gram-positive and Gram-negative non-fermenting bacteria at 40% and 5.52% respectively. This result was similar to those reported by Nagalo *et al.* in 2023 in Burkina Faso [24] and Wu *et al.* in 2024 in China [25]. *Staphylococcus aureus* predominated in blood-related infections, with a prevalence of 31.43%. It is considered one of the main causes of bacteremia in low- and middle-income countries [15] [26] and potentially fatal [27]. It is a germ that resides on the skin and in other parts of the body, such as the nose [28]. *Staphylococcus aureus*' ability to adhere to the skin of the human body facilitates host colonization [29] and establishment of complicated bloodstream infections [30]. The prevalence of *Escherichia coli* was 22.86%. It is a Gram-negative bacillus belonging to the *Enterobacteriaceae* family and is responsible for intestinal infections [31] or extra-intestinal infections such as blood-related infections [32]. Identification of local pathogens is important to ensure optimal patient care, as the choice of empirical treatment or prophylactic antibiotic therapy depends on the pathogens prevalent in a local community [33]. The sensitivity of oxacillin to *Staphylococcus aureus* strains was (18.18%). Clinical use of this antibiotic has led to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) [34]. Susceptibility to penicillin was (9.09%). This result was similar to that reported by Fernandez in 2020 in a study carried out in Kenya [35]. None of these *Staphylococcus aureus* strains were sensitive to vancomycin. Vancomycin is a first-line antibiotic used to treat patients with bacteremia caused by methicillin-resistant *Staphylococcus aureus* who are allergic to beta-lactams at a dose of 45 to 60 mg/kg per day [36]. Sensitivity to norfloxacin and levofloxacin was (9.09%), and to ciprofloxacin (27.27%). This is the result of overuse of antibiotics in hospital settings without a documented infection. Many of these antibiotics are also used in self-medication by communities who manage to obtain them from pharmacies and even without a prescription, leading to high antibiotic resistance and therapeutic impasse. Sensitivity to erythromycin was (2.73%). This result was lower than that reported in a study of patients hospitalized in the Czech Republic [31]. *Escherichia coli* strains showed a low sensitivity to amoxicillin of (12.50%). This prevalence is about half that reported by Ombet in Benin [37]. The high prevalence of resistance of *Escherichia coli* strains to beta-lactams, fluoroquinolones and aminoglycosides in our

study may be due to empirical first-line treatment of fever with a combination of several antibiotics.

5. Conclusions

Bacteremia are frequent, especially in patients with central or peripheral venous catheters, with a high frequency of *Staphylococcus aureus* and *Escherichia coli*. Antibiotic resistance of these isolates is very high in patients hospitalized at the Niamey General Reference Hospital.

We recommend an urgent review of the current policy of prescribing antibiotics in cases of suspected bacteremia in patients in Niamey city hospitals, in order to counteract the re-emergence of antibiotic resistance in bacteria and preserve antibiotics of last resort.

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Contributions of the Authors

All authors contributed to the design of the study, data analysis, writing and editing of the document and agreed to be responsible for all aspects of the work. All authors read and approve the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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